Supplemental Data

Synthesis of DOTA-DAPTA

The synthesis of DOTA-DAPTA was performed following standard procedures (*1*). The DOTA conjugated DAPTA was purified by solid-phase extraction (C-18 Sep-Pak cartridges, Waters) and RP-HPLC, respectively. The RP-HPLC elution gradient was from 100% H_2O to 65% acetonitrile in 45 min at a flow rate of 1.0 mL/min and UV absorption at 210 nm. The conjugation efficiency was about 98%, as determined by RP-HPLC.

Synthesis of Acetylene-DAPTA

DOTA-PEG-methacrylate, dithiolester radical addition fragmentation transfer (RAFT) agent, and 4-pentynoic n-hydroxy succinimide ester were prepared as previously reported (*2-4*). DAPTA (28.1 mg, 0.037 mmol) was dissolved in 2 mL anhydrous DMF and 4-pentynoic anhydride (25.1 mg, 0.128 mmol) dissolved in 1.5 mL anhydrous DMF was added dropwise to the solution and allowed to stir 2 days. Cold diethyl ether (15 mL) was added to the solution to triturate the product, which was subsequently dissolved in 2 mL of MilliQ water and freeze-dried (yield 31.0 mg, 70% pure, remainder starting peptide); MW (ESI) 836.39 {M+H+] (calc. 836.38).

Synthesis of poly(ethylene glycol) DAPTA methacrylate (DAPTA-PEGMA)

N_3-PEGMA (94 mg, 0.016 mmol) and Acetylene-DAPTA (30. Mg [~70% pure], 0.025 mmol reactive) were dissolved in a solution of 1.0 g DMSO and 0.65 g MilliQ water followed by the additions of 20 μL 5 wt% aqueous CuSulfate (0.0072 mmol) and 30 μL 5 wt% aqueous NaAscorbate (0.0064 mmol), respectively. The reaction was allowed to stir for two days with repeat additions of CuSO4 (50 μL) and NaAscorbate (75 μL) solutions after one day. The product was purified by washing (10x) with MilliQ water in 15 mL Centricon tubes (YM-5) and freeze-dried (yield 40 mg, 36%) (FT-IR, v (cm^{-1}): 3275, 2882, 1626, 1549, 1466,1359, 1341, 1279, 1240, 1146, 1099, 1060, 959, 841. GPC Mn 4200, PDI 1.10 (PEG standards, DMF). See supplemental info for ^1H NMR spectra.

Synthesis of DOTA-DAPTA-Comb

The synthesis of comb polymers was adapted from a previous report with the exception of poly(ethylene glycol) DAPTA methacrylate being incorporated into the polymerization mixture (*2*). To illustrate, PEGMA 5.0 kDa (123 mg, 0.025 mmol), DAPTA-PEGMA (17.4 mg, 0.0027 mmol), methyl methacrylate (MMA) (21.8 mg, 0.25 mmol), azobisisobutyronitrile (AIBN) (0.035 mg, 0.00021 mmol), DOTA-MA (11.1 mg, 0.016 mmol), and RAFT agent (0.16 mg, 0.00055 mmol) were dissolved in DMF (0.25 g). AIBN, DOTA-MA, and RAFT agent were added as DMF stock solutions. The solution was transferred to a 5 mL Schlenk flask and three freeze–pump–thaw cycles performed before being heated at 70 °C for 120 h. Following the polymerization, the solution was diluted with DMF, transferred to four 15 mL Centricon tubes (YM-50), and extensively washed with DMF, removal of monomers monitored by GPC. The copolymer was then washed with Milli-Q water (5x) and freeze-dried to give the desired graft copolymer as a white powder (yield 40 mg); (FT-IR, v (cm^{-1}): 2883, 1727, 1466 1359, 1342, 1278, 1241, 1146, 1098, 1060, 961, 841, 749. MW = 200 kDa, PDI 1.7 (PMMA standards, DMF).

Assembly of Nanoparticles

After removal of t-butyl protecting groups, the polymers were dissolved in DMSO (1 wt%), a rapid addition of an equal aliquot achieved assembly, and DMSO was removed by centrifugal filtration, resulting in particles of 14.8 nm and 20.4 nm (dynamic light scattering) for the targeting DOTA-DAPTA-Comb (zeta potential: -6.24 ± 2 mV) and non-targeting DOTA-Comb (zeta potential: -35± 2 mV) particles, respectively (Fig. S1).

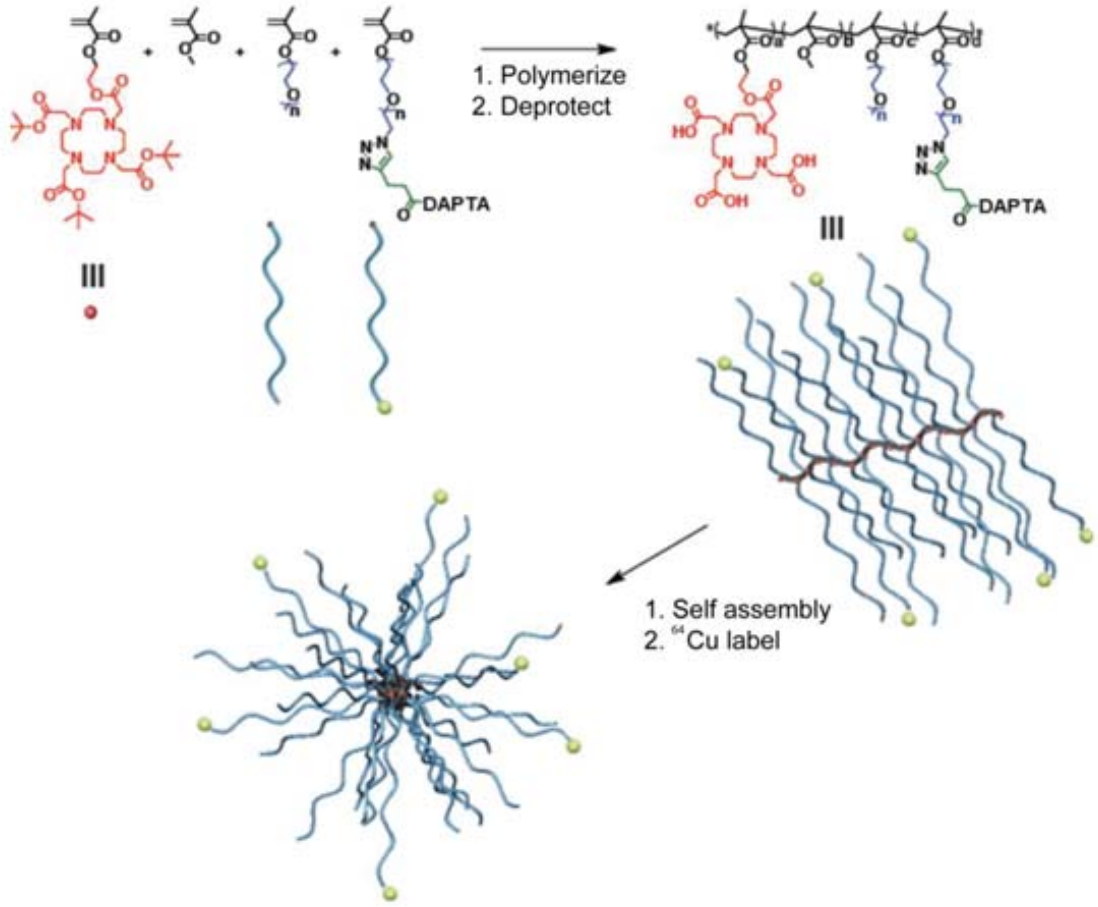
^{64}Cu Labeling of DOTA-DAPTA, DOTA-DAPTA-Comb, and DOTA-Comb

DOTA-DAPTA (7.5 μg, ~6 nmol) was labeled with ^{64}Cu (0.37 GBq) in 100 μL of 0.1 M ammonium acetate buffer (pH 5.5) at 43°C for 1 h, with a yield of 82.5% ± 6.8% and specific activity of 50.8 ± 6.1 MBq/nmol (n=5). DOTA-DAPTA-Comb and DOTA-Comb (5 μg, about 5 pmol) were labeled with 185 MBq of ^{64}Cu in 100 μL of 0.1 M, pH 5.5, ammonium acetate buffer at 80ºC for 1 h with a yield of 65.5% ± 5.3% (n=15) and 71.3 ± 4.6% (n=5), respectively. The ^{64}Cu-DOTA-DAPTA-Comb and ^{64}Cu-DOTA-Comb were purified by 2 mL Zeba spin desalting column after ethylene diamine tetraacetic acid challenge (10 mM in 50 mM, pH 7.4 phosphate buffer). The radiolabeling yields of the nanoparticles were

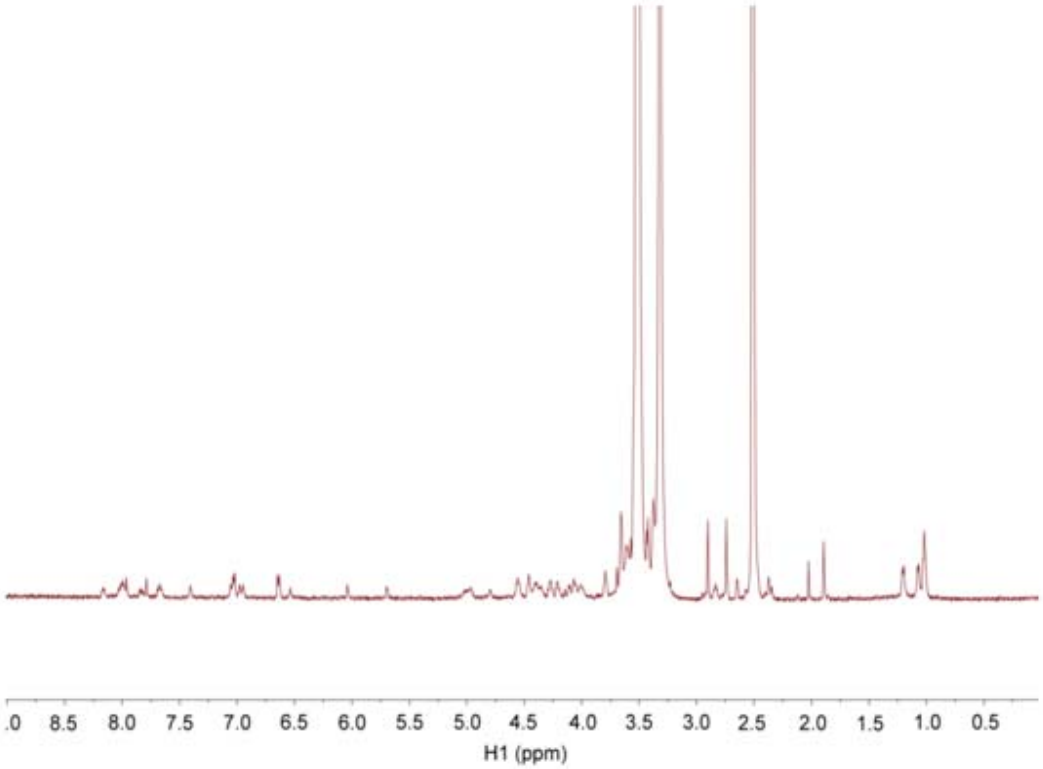
measured by radioactive thin-layer chromatography (Bioscan). The specific activities of ^{64}Cu-DOTA-DAPTA-Comb and ^{64}Cu-DOTA-Comb were determined as 2.14 ± 0.52 MBq/ pmol and 2.22 ± 0.30 MBq/ pmol, respectively.

Mouse Vascular Injury Model

Briefly, ApoE$^{-/-}$ mice at age of six weeks were fed a Western Diet (Harlan) (42% fat) for two weeks prior to the procedure. Each mouse was anesthetized with standard inhaled anesthetic protocol (1.5-2% isoflurane) by induction in a chamber, and maintenance anesthesia was administered via a nose cone. The ventral sides of the hind limbs were shaved and swabbed with Betadine/alcohol to create an aseptic region. An incision was made in the right femoral region by using a surgical microscope (up to 50 x). The femoral artery and vein were isolated using 6.0 silk sutures. Then the femoral artery branch was established using a small incision and was made into the vessel with micro-scissors. A 0.008 inch hydrophilic guide wire (Mirage, ev3 Endovascular Inc.) was inserted into the branch and advanced approximately 2 cm into the descending aorta and left there for 1 minute to induce endothelial injury. The guide wire was withdrawn and the branch was tied off. The femoral incision was closed with a 3.0 silk suture using interrupted surgical knots.



Supplemental Figure 1. Schematic representation of DOTA-DAPTA-Comb nanoparticle synthesis and assembly.



Supplemental Figure 2. ^{1}H NMR spectra of DATPA-PEG-methacrylate monomer.

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